Marking the 50th Anniversary of *Immunology*

# Special regulatory T-cell review: A rose by any other name: from suppressor T cells to Tregs, approbation to unbridled enthusiasm

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## Summary

**In the early 1970s a spate of papers by research groups around the world provided evidence for a negative regulatory role of thymus-derived lymphocytes (T cells). In 1971, Gershon and Kondo published a seminal paper in** *Immunology* entitled 'Infectious Immunological Tolerance'[1] indicating that such negative regulation could be a dominant effect that prevented otherwise 'helpful' T cells from mediating their function. Over the next decade, suppressor T cells, as these negative regulatory cells became known, were intensively investigated and a complex set of interacting cells and soluble factors were described as mediators in this process of immune regulation. In the early 1980s, however, biochemical and molecular experiments raised questions about the interpretation of the earlier studies, and within a few years, the term 'suppressor T cell' had all but disappeared from prominence and research on this phenomenon was held in poor esteem. While this was happening, new studies appeared suggesting that a subset of T cells played a critical role in preventing autoimmunity. These T cells, eventually dubbed 'regulatory T cells', have become a major focus of modern cellular immunological investigation, with a predominance that perhaps eclipses even that seen in the earlier period of suppressor T cell ascendancy. This brief review summarizes the rise and fall of 'suppressorology' and the possibility that Tregs are a modern rediscovery of suppressor T cells made convincing by more robust models for their study and better reagents for their identification and analysis.

**Keywords:** immunoregulation; regulatory T cell; suppressor T cell; T lymphocyte

## Introduction

As much as we would all like to think otherwise, the conduct of science is as subject to human foibles such as ego, prejudice, and emotional-driven belief as many other societal activities. While we strive for a goal of dispassionate assessment of data, we cannot avoid coloration of our views by these factors nor avoid the impact of group-think. The history of studies on negative regulation of immune responses by subsets of T cells is a prime example. A large intellectual edifice involving suppressor T cells (Tsup) was established over a decade of intensive investigation by major laboratories, only to crumble with the publication of a limited number of studies that raised questions about certain reagents and molecular analyses. Many of those active in this area abandoned the field and the reputation of those who persisted suffered greatly. Yet recent findings suggest that there was substantial truth to the basic phenomenology of T cell-mediated suppression and many of the observations made in these 'discredited' early analyses have been reprised in more modern studies of what are now called regulatory T cells (Tregs). Here I provide a brief overview of the rise and fall of Tsup and the reincarnation of these cells along with the theory of immunosuppression in the form of Tregs.

## Early days

In the late 1960s evidence emerged that adaptive immunity was the product of two major classes of lymphocytes, B (bone-marrow derived) cells that made conventional immunoglobulin antibodies and T (thymus-derived) cells that were responsible for reactions such as delayed-type


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hypersensitivity (DTH) and also involved in co-operation with B cells for generation of high-affinity antibody responses[2–6]. Within a short time, studies by several laboratories showed that T cells not only had these effector and positive co-operative roles, but also could depress immune responses[1,7–16]. Early thoughts of mechanism revolved around B-cell destruction, but a seminal 1971 paper on infectious immunological tolerance by Gershon and Kondo[1], suggested that negative regulation could be mediated by interference with the activity of otherwise positive acting T cells. In concert with the emerging evidence for subsets of T cells, in particular as defined by anti-Ly antisera[17–25], this led to the notion that suppressor T cells were a specialized subset of lymphocytes whose role was to limit immune responses[26,27].

A confluence of rapidly emerging experimental evidence and the powerful logic of the argument that the immune system needed suitable brakes to prevent excessive activity led to rapid acceptance of suppressor T cells as a key paradigm of the growing field of cellular immunology. In short order, a number of experimental models involving proteins, red blood cells, haptens, and tumours (reviewed in refs[28–38]) were reported, all of which showed evidence of the activity of Tsup. Genes in the major histocompatibility complex (MHC), already known to control the positive aspects of immunity mediated by T cells, were found to also control suppressor T-cell function[39–41].

Research using a variety of antisera began to complicate the initial picture of Tsup function. An alloantiserum generated to a previously unrecognized subregion within the MHC, the so-called I–J subregion, was reported to react with Tsup and more strikingly, to be bind a soluble material derived from Tsup (TsF) that could substitute for intact cells in mediating negative immune regulation *in vitro* and *in vivo*[42–44]. Other antisera to specificities called Lyt1 and Lyt2 reacted in a differential manner with Tsup in different laboratories, a paradox whose solution was suggested to be the need for two distinct T-cell subsets to interact with each other for suppression to be manifest[45–48].

The field became even more baroque under the influence of the contemporaneous paradigm of isotypic guidance of immune function[49]. Anti-idiotypic antibodies made against immunoglobulins of a given specificity were claimed to recognize Tsup and TsF with a similar specificity and immunoglobulin H (IgH) allotypes were reported to provide another level of genetic restriction to the interactions involved in suppressive function[50–56].

As these data on multigenic control emerged and other data were reported indicating that communication among the T-cell subsets involved in suppression could be mediated by a variety of distinct TsFs, some of which showed both specificity for antigen and genetically restricted interactions, questions grew about whether the discordant nature of the complex models in the different experimental systems could be reconciled. Several attempts were made to provide an overarching theory of Tsup function (see ref. 34 for one such effort), most of which were based on the notion that different laboratories were interrogating distinct parts of a long concatenation of cell–cell interaction events that ended in the production of an antigen-unspecific effector factor that interfered with antigen-presenting cell function. However, larger problems loomed as molecular biology and monoclonal antibody technology began to bring a new rigour to the Wild West of immunological investigation of cell subsets and soluble mediators.

## The beginning of the end

These complications involving many cell types and genetic restrictions notwithstanding, suppressor T cells remained an accepted part of the immunological firmament in the early 1980s. However, as molecular cloning began to have a major impact on biological studies in many fields, including immunology, and as the technique of Köhler and Milstein[57] began to yield monoclonal replacements for complex and not always reproducible or specific antisera, matters became problematic. Among many events that led to a loss of confidence in the Tsup story as it existed at this time, several stand out. One was the reported purification of an antigen-specific TsF[58], only to have another paper provide evidence that what had been identified was an apolipoprotein, not an antigen-specific T-cell derived molecule[59]. Another was the analysis, first at the mRNA level[60] and then by genomic sequencing[61], of the putative region in the MHC encoding I–J. No specific transcripts corresponding to this region were observed in Tsup and no nucleotide polymorphisms were found in DNA from the two strains of inbred mice [B10.A(3R) and B10.A(5R)] that were used to produce the initial alloantisera that identified I–J. An attempt was made to explain these data by postulating that I–J was actually an anti-idiotype to the antigen-specific component of Ts and TsF[62–64], which would result in the apparent mapping of I–J to the MHC because of the influence of such gene products on the combining site of the clonotypic receptors of T cells. However, this 'solution' of the I–J problem gained little traction. Finally, no specific T-cell receptor (TCR) β chain rearrangements were seen in DNA from putative Tsup hybridomas[65], although this result was less than definitive in that γδ T cells also lacked such rearrangements in many cases.

Taken together, this spate of negative findings using the most modern tools for immunological investigation threw cold water on the entire field of Tsup and TsF. From a

field with great cache that dominated international meetings for years, it rapidly acquired a taint that impacted publication of papers in the area and perhaps even more significantly, the capacity of those heavily invested in the study of Tsup to maintain research funding. The result was a rapid loss of momentum and the development of the belief that many if not most of the observations reported in the area were flawed if not of dubious provenance. Citations for Tsup peaked as these events were unfolding and then rapidly declined to almost none.

## The rise of Tregs

Like a phoenix, negative regulatory T cells rose from these ashes to a position of prominence in today's immunological thinking over precisely the interval from the demise of Tsup to the present. Insightful studies of autoimmunity arising in mice thymectomized early after birth suggested that a subset of T cells was critical for the restraint of effector development/function among more conventional T cells (reviewed in ref. 66). Close on the heels of these observations, other laboratories showed that transfer into immunodeficient hosts of purified T cells lacking activation/memory markers led to autoimmunity, especially inflammatory bowel disease, and that addition of a subset of T cells with certain memory cell markers could prevent this disease in a dominant manner[67,68]. A report that inhibitory T cells were marked by high CD25 expression[69] and the demonstration that such cells limited TCR-induced T-cell proliferation of conventional CD4 and CD8 T cells in culture[70] opened up the field to study by the larger community, which rapidly confirmed that the memory/effector phenotype cells identified using CD45 and CD25 markers (Tregs) were important to preventing autoimmunity in lymphopenic animals given small numbers of conventional T cells and could also limit anti-pathogen immunity in certain cases (reviewed in refs 71–74).

The field made a major jump forward with the recognition that the transcription factor FoxP3 was critical for the development and/or function of these inhibitory Tregs[75–77] and that either genetically modified reporter animals[77–79] or monoclonal antibodies to FoxP3 could be use to identify these cells with some assurance. Perhaps most convincing that these Tregs played a major role in immune homeostasis was the dramatic autoimmune phenotype of mice with a mutation in the FoxP3 gene (scurfy mice) and the analogous autoimmune disease seen in humans with mutations in the FoxP3 gene (IPEX)[80–83].

Citations of papers describing Tregs is now rising in a manner akin to that seen in the early days of Tsup, and these days no broad-based immunological meeting worth its salt lacks a major session on these cells. The intense interest in this area of research is clearly evidenced by the fact that such sessions are routinely oversubscribed, with attendees spilling out the doors of the lecture hall.

## Are Tregs really Tsup in disguise?

There is little question that a distinct subset of T cells, most of which originate in the thymus, contribute to effective immune homeostasis. Whether all the claims made for a role of these so-called natural or nTregs in immune functioning will prove out in the long run is unclear, but their importance seems beyond question. Why have these cells been embraced so readily given the odious nature of Tsup, are the two types of inhibitory cells related, and what should the Treg field be careful about, given past history?

As to the first issue of acceptance, in contrast to the ephemeral nature of I–J, Tsup hybridomas, DTH measurements on mouse ears, and the like, Tregs are readily identified by widely available monoclonal reagents to CD25 and FoxP3, as well as by knock-in reporter mice expressing GFP in cells that transcribe the FoxP3 locus. Thus, many laboratories can study these cells with confidence that they are looking at more or less the same cell population (although some level of smug absoluteness is creeping into thinking on this issue and poses a serious risk going forward). Furthermore, using these tools, consistent data on the role of this subset in limiting autoimmune responses *in vivo* have emerged from a very large number of independent laboratories studying diverse model systems. In contrast to I–J on Tsup, there certainly is no question about the reality of CD25 and FoxP3 expression by cells that, as a population, can mediate negative immunoregulatory effects. For these reasons, the problems of the past that related to Tsup are not considered relevant to Tregs, at least in terms of their identity and reality.

With respect to whether Tregs and Tsup are related, this is a question that perhaps only those of us involved in the original Tsup work spend time considering. But it is instructive, I think, for a newer generation to appreciate that many of the observations made with regards to Tsup are strikingly similar to data relating to Tregs, and that even some of the most controversial of issues concerning Tsup are difficult to dismiss in light of modern knowledge and what was known (or more importantly, not known) when these early findings were reported.

1. An often reported characteristic of Tsup is that they could not be cloned *in vitro* using methods that reproducibly led to creation of lines and clones of conventional CD4 and CD8 T cells; this property is entirely consistent with the well-accepted anergic character of Tregs *in vitro*[71,73,74] and the difficulty of driving their proliferation in culture except with heroic concentra-


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tions of interleukin-2 (IL-2) and strong stimulation with anti-TCR and CD28 antibodies.[84]

2. The *in vivo* function of Tsup was eliminated by treatment with low dose cyclophosphamide[85]; this has also been reported to be true for Treg function.[86]
3. Natural Tregs are CD4 T cells that are selected in the thymus by recognition of MHC class II molecules[71,73,74,77] and that function in the periphery as do other CD4 T cells, using MHC class II molecules for antigen recognition; likewise, the genes regulating Tsup function were mapped to the class II region of the MHC.[40,41]
4. The antigen recognition unit of T cells is a disulfide-bonded heterodimer with two chains in the ~40–50 000 MW range[87]; reports on the molecular nature of the antigen-specific suppressor factor of Tsup characterized the material as a disulfide-linked heterodimer of similar molecular mass[88,89]; it would be quite fortuitous for the authors of the latter work to have arrived at this result by chance, given that only immunoglobulins of much greater molecular mass were known to be antigen-specific molecules at the time.
5. The lack of TCR β rearrangements in Tsup hybridomas involved studies of DNA from long-term cultures of these notoriously unstable cells, without repeated selection for antigen-specificity and without the possibility of sorting for expressed TCR because the relevant antibodies were not available; selection of $CD3^+$ cells from such cultures once the proper reagents became available showed that these cells did express conventional TCR and that increasing the proportion of $TCR^+$ cells from a few percent to close to homogeneity also increased suppressive activity by a comparable extent[90]; likewise, authentic TCR αβ determinants were found on TsF from Tsup when the proper monoclonal reagents became available.[91]
6. The ability of Tsup and TsF to bind antigen in the absence of MHC class II molecules is seemingly problematic given our knowledge of T-cell receptor structure-function and recognition of peptide–MHC molecule ligands; however, several reports have shown that T cells with a functional requirement for antigen presentation by MHC class I or II molecules have TCR that, when isolated biochemically, can show direct binding to certain antigens independent of MHC molecules, as claimed for Tsup and TsF.[92–94]
7. Infectious tolerance has been rediscovered[95,96] and is now an increasingly popular view of how Tregs work; although the mediator of this infectious process is considered to be an antigen-unspecific cytokine, the idea that the potency of suppression is amplified by recruitment of new cells into the suppressive pool parallels the Ts1-Ts2-Ts3 schemes from the old Tsup days, as does the evidence that the ultimate mediation of Treg activity involves any of several immunosuppressive cytokines (transforming growth factor-β (TGF-β) and IL-10 chief among these[71,73,74,97]) that exert their activity via antigen presenting cells, just as concluded in the early Tsup studies.[98]

Finally, what lessons should be drawn from the rise and fall of Tsup and the recent impact of Treg studies? At a minimum, given the list above, it seems that the field threw the baby out with the bathwater. Many of the biological observations made in connection with Tsup seem quite robust and similar if not identical to those now being found true of Tregs. The major problem in the earlier work was the inability of those involved to move the field forward on the molecular level and the possibility that some key observations were misleading, if not just plain wrong. But do any of us imagine that all the aspects of the biology of Tregs that are well accepted today are absolutely correct? Already questions are being raised about whether the widely used *in vitro* assay for suppression of proliferation is related more to competition for cytokines than to the better accepted mechanisms mediating the *in vivo* physiological function of these cells[99]. Likewise, the cell–cell contact requirement for suppression claimed in the culture assay may not be what it seems in terms of real Treg function[100,101]. Early reports of Treg function being entirely divorced from TFG-β activity[102,103] are being reconsidered in light of strong data to the contrary (reviewed in ref. 97), but this change in viewpoint has not resulted in a push to discredit all the work on Tregs done prior this point in time. The absolute linkage of FoxP3 expression with Tregs has been called into question by data from human cells, which express this protein without necessarily showing the *trans*-regulatory properties of mouse Tregs[104]. Yet the field does not question the essential role of FoxP3 in Treg development and function. Finally, even in the mouse, a debate now rages about the occurrence and relevance of induced Tregs[105], but this is a more typical scientific dispute within a field that accepts the underlying existence of regulatory cells expressing FoxP3.

In other words, the fact that errors of interpretation and inadequate experiments exist in the Treg arena, just as they did in the Tsup era, has not done in the field in the way that a few reports disputing aspects of the 'received wisdom' in the Tsup field did 25 years ago. Perhaps we have all grown up a bit since then and have a more sophisticated view of the complexity of the immune system and our limited capacity to get everything right in every report probing its workings. Possibly, the field has also learned a lesson from the self-inflicted wound of the early 1980s when the results of serious scientists were discredited without adequate consideration of alternative interpretations for the experimental data, and without seeking to separate the wheat from the chaff.

Indeed, if more effort gone into separating the widely reproduced and fundamental aspects of Tsup function

from less certain claims introduced by limited technology and suboptimal experiments, we might be even further along than we are in understanding immune regulation. At the very least, immunology would not have given itself such a black-eye in the view of other biologists and we would have less explaining to do about whether we pursue our work with the rigor of other fields. Some of the same internal derogation of other investigators in the field is beginning to creep into the Treg arena – it seems better for us all to carefully question potentially incorrect conclusions on their merits and use our concern about these matters to drive better experiments, than to engage in the type of self-defeating internecine warfare that led to the demise of the Tsup field in the past.

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